THE RESULT OF IMPROVED NUTRITION ON THE LANSING EFFECT IN *MOINA MACROCOPA*

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In a previous study (Murphy, 1970) it was noted that *Moina macrocopa* lived longer if the monoxenic culture medium contained a greater variety and larger amounts of water soluble vitamins.   Similar effects of vitamins on the life span of other cladocera have been reported (Flückiger and Flück, 1950; Fritsch, 1953). Because *Moina macrocopa* is exceptionally short lived (Papanicolau, 1910; Wood and Smith, 1932) and therefore convenient for studies of aging, it was decided to investigate whether or not the Lansing Effect (Lansing, 1947) could be demonstrated with this species, and if so to find out if the extinction of old orthoclones could be prevented by improved nutrition.

The Lansing Effect refers to the finding that clones of a rotifer, *Philodina citrina*, raised through successive generations from old mothers, died out.   The lines, which Lansing named orthoclones, were formed as follows.   A fifth orthoclone, for example, would be started by isolating the young rotifer ($5F_1$) born of a mother on her fifth day of life.   When this daughter rotifer is five days old, its daughter ($5F_2$), born that day, would be isolated.   This process is repeated to produce $5F_3$, $5F_4$, *etc.*   Thus, every animal in an orthoclone was born when its mother was the same age.   In a fourth orthoclone all the mothers were four days old; in a tenth orthoclone, ten days old.   Lansing showed that there were important differences between the young orthoclones and the old orthoclones.   While the young orthoclone continued indefinitely in the culture conditions used, all orthoclones formed after the animals stopped growing eventually became extinct (Lansing, 1948).   The older orthoclones displayed increasingly shorter life spans and an increasing tendency to die out.

Efforts to elicit the Lansing Effect in other organisms have met with failure. Comfort (1953) could not demonstrate any life shortening in 30 day orthoclones of *Drosophila subobscura*, and Fritsch (1956) could not demonstrate the effect with *Daphnia magna*, although he did not make an effort to test the oldest orthoclones.

Because each generation of an old orthoclone must be kept for virtually the entire life span of the animal before the next generation is born, studies of this type can, in practice, only be performed on animals with very short life spans, such as *Moina macrocopa*.

Our results show that there is a very striking Lansing Effect with *Moina macrocopa* and further that it can be modified by treating the older orthoclones with relatively high concentrations of inositol or liver infusion.

Methods and Materials

The monoxenic culture procedure used has previously been described in detail (Murphy, 1970). The medium is shown in Table I. Note that one component, pyridoxal, was omitted in the original description. All experiments were done at 20° C. Supplementary vitamins were made up at 10-fold concentrations, sterilized by filtration through a 20 micron porcelain filter cylinder and added directly to the petri dishes containing 9 ml of filtered medium. Dehydrated liver infusion (Oxoid) was made up 0.7 gram/100 ml, autoclaved and 1 ml, including some undissolved particles, was added per dish. The food organism was *Chlamydomonas reinhardii*, Indiana U. strain #90 grown in the medium of Levine and Ebersold (1958) enriched with one gram per liter of N-Z-Case peptone (Sheffield Chemical Co.) and with agar omitted. The animals were transferred three times per week.

Fritsch (1956) when using *D. magna*, which produced a clutch of eggs every two days, defined an orthoclone on the basis of clutch number. This is convenient and

Table I

Composition of the medium (mg/liter). For directions on how to make up this medium, see Murphy (1970)

Calcium acetate · X H_2O	59.0
Potassium penicillin (U.S.P.)	215.0
Streptomycin sulfate (U.S.P.)	7.0
Bovine albumin, fraction V (Armour)	67.0
Calcium pantothenate	7.0
B_{12}	0.0003
Thiamin	0.6
Riboflavin	0.4
Nicotinamide	1.3
Folic acid	3.3
Biotin	0.3
Putrescine	0.3
Choline	5.0
Inositol	11.0
Pyridoxal	5.0
Hutner's Trace Elements	0.3 ml.

possibly more meaningful than chronological age when referring to cladocera (Ingle, Wood and Banta, 1937). We have followed the same terminology.

Moina macrocopa, under the basic conditions used in this study, is a highly variable animal. It usually lives about ten days to two weeks and produces about 100 parthenogenetic young divided into five clutches. Even though some animals may die at any time as if a random death process were operating, under improved nutritional conditions, the number of deaths in all age groups is diminished. As there is no apparent way to distinguish between the causes of death in younger and older animals, all deaths are included in the data. This makes larger experimental samples necessary to reach statistically significant levels, but the overall conclusions are not affected.

Animals chosen for transfer have been selected on the basis of the largest clutches from the most actively moving adults. This would be expected to
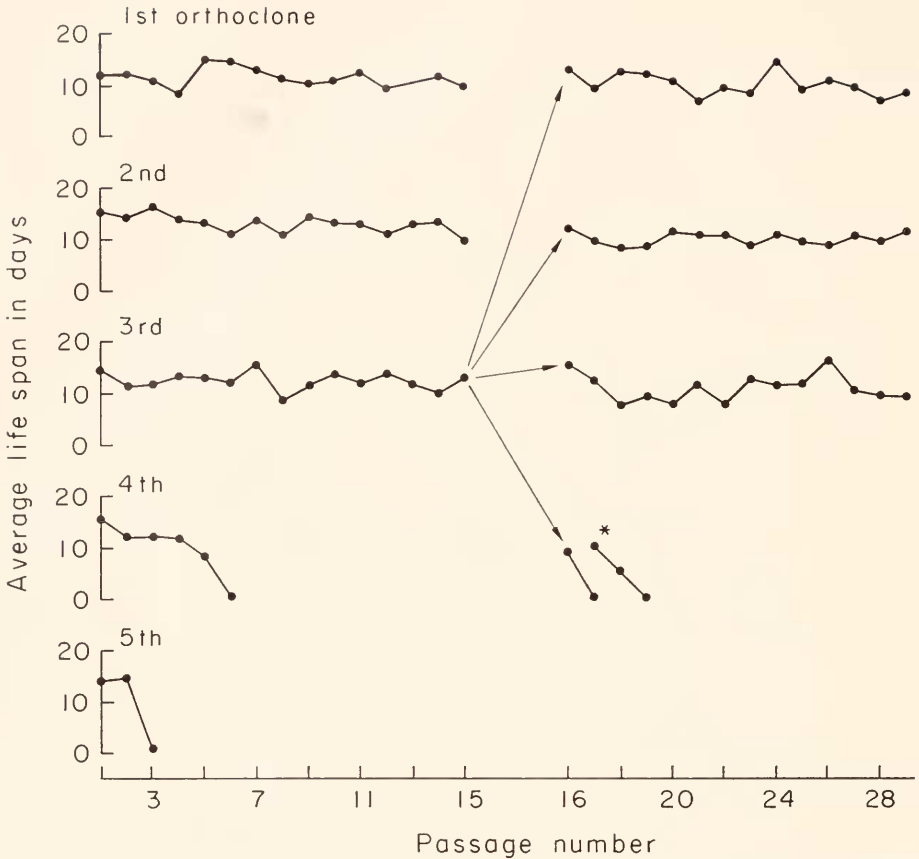
FIGURE 1. The average life span (five animals/datum) of successive passages of the first through fifth orthoclones of *Moina macrocopa*. The fourth and fifth orthoclones die out thus demonstrating the Lansing Effect. At passage 15 of the third orthoclone, the experiment was restarted using the progeny of one animal. The animals indicated in the datum marked * were the young of a mother raised with extra inositol.

eventually yield a clone superior to the original but no such effect was found during the limited time span of this study. On the contrary, it is necessary to select the strongest animals to maintain any clone in continuous passage.

RESULTS

Demonstration of the Lansing effect in Moina macrocopa

Figure 1 shows the average life spans in days of orthoclones from the first through fifth clutches. Six clutches are rare under our conditions. The data are shown as averages of five animals, but it should be noted that nearly every datum contains animals that died after 4 to 5 days or animals that lived 16 to 18 days. In spite of the variation it is clear that the first, second and third

TABLE II

The effect of inositol on the average life span and average number of young per lifetime on fourth orthoclone Moina macrocopa. (*Each datum represents the mean of five animals*)

	Inositol concentration (mg/liter)	Passage 1		Passage 2		Passage 3	
		Life span	Young	Life span	Young	Life span	Young
Control	11	5.6	4.8	died out			
Inositol pulsed*	61 or 11	7.9	46.5	13.6	91.6	9.5	85.0
Vitamin mixture pulsed*†	61 or 11	10.0	49.8	11.0	50.8	—	—
Inositol continuous	61	9.9	42.2	15.2	69.6	9.4	51.4

* Pulsed indicates that the animals were in the stronger concentration for 24 hours twice a week.
† This group had extra riboflavin, folic acid, calcium pantothenate and nicotinamide along with inositol.

orthoclones were definitely established at the end of the experiment while the fourth and fifth died out.

The slight apparent decline in life span shown by the first and second orthoclones is an artifact and did not continue. The third orthoclone has been continued for over 28 generations with no diminution of life span, average number of young produced, or apparent vigor.

The effect of inositol and some water soluble vitamins on old orthoclones

In a previous study (Murphy, 1970) it was noted that shortening of life span due to avitaminosis did occur in the daphnidae possibly because the biosynthetic mechanism leading toward egg production is capable of drawing so heavily on its reserves that the animal is thrown into pathological deficiency. We had also noted that species that could barely survive under the conditions being used were the best indicators of an improvement in the medium because they quickly showed such sharp endpoints as living or dying, or bearing or not bearing young.

On the basis of these previous experiments it was decided to test fourth orthoclone animals with higher concentrations of some of the components of the medium. In a preliminary experiment, a mixture of five was chosen and used at a concentration of five times that in the regular medium.

The first substances tried were inositol, riboflavin, folic acid, calcium pantothenate and nicotinamide. The initial studies showed that the mixture was toxic after 24 hours. For this reason animals were treated with 24 hour pulses twice a week. On this regime, the fourth orthoclone became established. A preliminary experiment indicated that inositol alone was responsible for the result. Table II lists the data for life span and number of young for three orthoclone passages under several nutritional regimes. There is no significant difference among the groups with added inositol except that the number of young produced after two 24 hour treatments each week shows a consistently higher average. Although the difference is significant only at the $P = < 0.2$ level, this routine was chosen because we felt the animals also looked more vigorous.
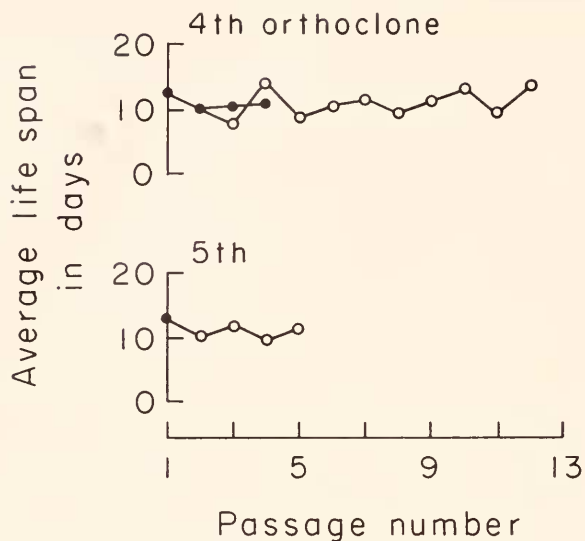
FIGURE 2. The average life span (five animals/datum) of successive passages of the fourth and fifth orthoclones of *Moina macrocopa* after extra inositol was added to the medium for 24 hours, twice a week. This figure should be compared with the fourth and fifth orthoclones in Figure 1. ●———● indicates that the medium also contained extra amounts of vitamins; ○———○ indicates no extra vitamins.

The pulses of inositol at 61 mg/liter for 24 hours, twice a week, alternated with a concentration of 11 mg/liter the rest of the time. As can be seen from Figure 2, the fourth and fifth orthoclone could now be established.

Test of seven more vitamins on the fifth orthoclone

Using the same arguments as those used in previous experiments, pulses of the remaining six water soluble vitamins (Table I) at five times the concentrations used in the regular medium and a water soluble form of vitamin E (DL-α-tocopherol phosphoric acid ester, disodium salt) 1 mg/liter were tried singly and in combination on fifth orthoclone animals already being supported by biweekly pulses of inositol. The results showed no consistent increase in life span over inositol alone. The fifth orthoclone's existence was extremely tenuous since generally only one of the five animals lived long enough to produce a fifth clutch of young.

Effect of liver infusion

Provasoli, Conklin and D'Agostino (1970) found liver infusion (Oxoid) to have an enhancing effect on *Daphnia magna* and *Moina macrocopa*. The concentration suggested by Douglas Conklin, Haskins Laboratories (personal communication) of 700 mg/liter added to the regular medium produced a most remarkable and almost immediate improvement in the fifth orthoclone cultures (Table III). The animals became more active and more deeply pigmented. The number of young born in a lifetime sharply increased and the females had their clutches of young a

TABLE III

The effect of liver infusion (Oxoid) 700 mg/liter on the fifth orthoclone of Moina macrocopa. *(Each datum represents the mean of five animals)*

	Passage 1		Passage 2	
	Life span	Total young*	Life span*	Total young*
Inositol pulsed 11–61 mg/liter	11.4	82.2	10.8	94.3
Liver infusion continuous	12.0	126.7	12.5	136.2

* The values for liver infusion are all significantly greater than those for inositol at the $P < 0.05$ level except for the lifespan values in passage 1. The average young/hatch in liver infusion is 24.9.

day earlier than the pulsed inositol controls. Although the life span did not markedly increase, the fifth orthoclone appears to have become solidly established because the young were born earlier.

Our preliminary results with a sixth orthoclone in liver infusion give little hope for its survival.

DISCUSSION

The interpretation that the Lansing Effect in *Moina macrocopa* is brought about by relative nutritional deficiency seems quite clear. It is tempting to compare the conditions which Lansing (1947) used to culture rotifers and speculate as to whether he too was observing a nutritional effect.

Lansing used a single species of alga, *Chlorella vulgaris*, as a food organism, culturing the rotifers in a mineral medium without added nutrients and transferring them daily. Probably some bacteria were present, but it is doubtful if they could have reproduced well enough in this environment to become a significant supplemental food source. Experiments with *Daphnia pulex* indicate that *Chlorella pyrenoidosa* or *Chlamydomonas reinhardii* alone are not adequate as food organisms (Taub and Dollar, 1968). Single species of algae may also be inadequate for rotifers. This suggests the possibility that Lansing's experiments may have been carried out under suboptimal nutritional conditions.

Whether the Lansing Effect has the same mechanism in rotifers as it does in a crustacean does not alter the fact that there exists in both organisms a nongenetic, transmissible, cumulative premature aging phenomenon operating over many life spans.

Other examples of non-genetic parental influence on life span have been recorded. Papanicolau (1910) observed a gradual reduction in life span of first orthoclones of *Moina rectirostris* and *Simocephalus vetulus* in the laboratory. Rockstein (1957) has shown that old houseflies produce young with short life spans, a result indicating that a Lansing Effect might have been operating. His animals were raised initially on sugar, milk and yeast and switched to sugar alone when adults. Recently, Zamenhof, van Marthens and Grauel (1971) have observed in rats that protein restriction one month before mating and continued through pregnancy reduces total brain DNA in the young. This defect is transmitted to the F_2 generation even though nutritionally adequate diets were reinstituted at the time

of birth of the F_1. The term "cryptic malnutrition" is used to describe the phenomenon.

The question of whether a further major increase in life span might be possible with *Moina macrocopa* remains open. The most productive and long-lived animals reported by Wood and Smith (1932) are very similar to our best in liver infusion. Stuart and Banta (1931) and Stuart, McPherson and Cooper (1931) report an average clutch size of 17 or less, lower than that found in our study (24.9 young/clutch). Terao and Tanaka report shorter life spans (1930) and lower reproduction rates (1928) at $20°$ C. However, the preliminary finding that the sixth orthoclone will not survive should probably be considered evidence that our animals are still not under ideal nutritional conditions. At any rate, the Lansing Effect has not been abolished. It is possible that the lack of a detectable Lansing Effect in Comfort's (1953) experiments with *Drosophila subobscura* was the result of the ideal nutrition of his cultures.

Finally, the finding that the Lansing Effect is not confined to rotifers raises the possibility that it may underlie the not uncommon experience of having cultures of various kinds fail after numerous apparently successful passages.

SUMMARY

Lansing showed that clones of rotifers raised through successive generations from old mothers will die out (Lansing Effect). We have found that the phenomenon also can be shown in a crustacean, *Moina macrocopa*, cultured monoxenically on *Chlamydomonas reinhardii*. Furthermore, we have found that the Lansing Effect can be modified and partly prevented by exposing the animals to relatively large amounts of inositol or liver infusion. It is suggested that the Lansing Effect in *Moina macrocopa* is the result of a long range, transmissible, cumulative, premature aging phenomenon due to relative nutritional deficiency.

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